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Serum HBeAg Quantitation During Antiviral Therapy for Chronic Hepatitis B

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Hepatitis Be antigen (HBeAg) seroconversion is considered the principal short-term goal of antiviral therapy in chronic hepatitis B. To test whether the pre- and per-treatment HBeAg quantitation has a higher predictive value than that of hepatitis B virus DNA (HBV-DNA) quantitation for the outcome of antiviral therapy in chronic hepatitis B. A quantitative measurement of HBV-DNA and HBeAg (AxSYM HBe 2.0 Quantitative, Abbott Laboratories) was undertaken in serial serum samples from 30 patients with 16-week interferon-α (IFN-α) treatment (follow-up 36 weeks; 14 responders) and from 15 patients with 24-week lamivudine treatment (follow-up 24 weeks; 2 responders).

In the group of interferon-treated patients, the median pretreatment HBV-DNA level was significantly lower in responders compared to non-responders ($P = 0.02$); the difference in median HBeAg level was not significant. However, the percentage of response was significantly related ($P = 0.003$) to the magnitude of decline in HBeAg level between the start of therapy and week 4. This phenomenon was not observed for HBV-DNA. Using multivariate analysis, it was found that the fall of HBeAg levels between weeks 0 and 4 was the most important independent predictor of response. In the group of lamivudine treated patients, the rapid decline in HBV-DNA (>90%) in 12 patients at week 4 had no relation to HBeAg seroconversion. In contrast, the fall in HBeAg-level (one patient with >50% reduction at week 4 seroconverted) appears to be predictive. Quantitation of HBeAg at start and early during therapy may have clinically important predictive value for long-term response to antiviral therapy. ***J. Med. Virol. 53:282–287, 1997.***

KEY WORDS: hepatitis B virus; HBeAg; quantitation; antiviral therapy; interferon; lamivudine; prognosis

INTRODUCTION

Successful antiviral therapy in chronic hepatitis B with active wild-type viral replication is characterized by (HBeAg) clearance from the circulation. In most cases, the disappearance of HBeAg is followed by the development of anti-HBe antibodies in a process denoted as HBeAg seroconversion. An early marker and prerequisite for HBeAg seroconversion is the disappearance of serum (HBV-DNA); however, disappearance of HBV-DNA is not always followed by HBeAg seroconversion. The level of HBV-DNA in pretreatment serum is a prognostic factor in predicting response to interferon (IFN) therapy [Brook et al., 1989; Perrillo et al., 1990]; furthermore, monitoring patients for quantitative HBV-DNA during therapy is thought to be useful to determine nonresponse or the need for prolonged therapy.

Quantitative assessment of HBeAg in pretreatment serum and during therapy might be a good alternative, since HBeAg seroconversion is the goal of therapy and disappearance of HBV-DNA does not always predict HBeAg seroconversion. The value of quantitative HBeAg measurement was suggested in studies assessing IFN therapy [Berk et al., 1992; Perrillo et al., 1993], especially for those patients who may benefit from prolonged therapy [Janssen et al., 1992].

Until recently, quantitation of HBeAg was cumbersome and not well standardized. The development of kinetic assays that are widely used in clinical laboratories now permits precise quantitation when used in conjunction with an international reference standard. This study illustrates the potential of quantitative measurement of HBeAg, in comparison to HBV-DNA for the prediction of outcome of antiviral therapy.

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METHODS

Study Groups

Serum samples that had been frozen for 1–5 years were available from the following categories.

Control Groups

1. Prevaccination serum samples from medical students (n = 102).
2. Serum samples from chronic hepatitis B patients negative for HBeAg and positive for anti-HBe; normal or low level AST (n = 50).
3. Series of dilutions of four HBeAg-positive sera. Five dilutions were assayed in six runs on different days for determination of inter-assay variability; two samples were tested seven times in one run to determine intra-assay variability.

Treatment Groups

1. Serial serum specimens from 30 patients participating in a study of IFN therapy (10 million units 3×/week for 16 weeks; 14 responders, 16 nonresponders; follow-up: 36 weeks). Serum samples were taken before start (two samples within 8 weeks) of therapy, during and after therapy at 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 44, and 52 weeks from start.
2. Serial specimens from 15 patients participating in a dose response study on lamivudine (25, 100, and 300 mg/day for 24 weeks, and follow-up of another 24 weeks). Serum samples were taken before start (two samples), during and after therapy at 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48 weeks from start.

These studies were approved by the local Medical Ethical Committee and written informed consent was given by all patients.

Laboratory Methods

Quantitative HBeAg measurements were carried out by AxSYM (AxSYM HBe 2.0 Quantitative, Abbott Laboratories). AxSYM is a fully automated random-access microparticle enzyme immunoassay system. The assay is based on two different monoclonal antibodies and its reference preparation for quantitation of HBeAg makes use of purified recombinant HBeAg. The internal calibration panel is standardized with the Paul Ehrlich Institute (PEI) standard preparation for HBeAg. Results are expressed in PEIU/ml after comparing the rate of fluorescence for a specimen with a standard curve composed of the six samples of the internal reference containing 0, 0.25, 3, 30, 60, and 120 PEIU/ml, respectively. The cutoff level of the assay was set at 0.25 PEIU/ml.

Serum samples were tested undiluted after centrifugation for 15 min at 20,000*g* in an Eppendorf centrifuge or without centrifugation in a 1:50 dilution after predilution by hand with the AxSYM diluent. Samples that have been frozen and thawed were centrifuged; lipidic materials were avoided to prevent false reactivity. In a limited number of cases the internal (1:50) dilution feature of AxSYM was used for undiluted and manually prediluted serum samples, if initially obtained results were out of the calibration range (>120 PEIU/ml) and sufficient serum was available.

For determination of the inter-assay variability, four serum samples were diluted in fetal calf serum. Diluted samples were prepared once for the six runs and frozen until use.

HBV-DNA was determined by HBV-DNA assay (Abbott Laboratories). Qualitative measurements of HBV parameters were carried out by IMx assays (HBsAg, anti-HBc, anti-HBs, HBeAg, and anti-HBe) (Abbott Laboratories) or radioimmunoassays (anti-HBc, anti-HBs, HBeAg, and anti-HBe) (Abbott Laboratories).

Definition

Response to therapy is defined as a simultaneous negative result for HBeAg (<0.25 PEIU/ml by AxSYM) and HBV-DNA (<3 pg/ml by Abbott HBV-DNA assay) in two consecutive samples during therapy or follow-up evaluation.

Statistics

Least-square regression lines were calculated to assess the relation between the level of logarithmically transformed HBeAg measurements and the twofold dilution steps for four individual HBeAg-positive sera. Multiple regression analysis was used to compare these slopes.

Continuous and categorical variables were compared between groups, using, respectively, the Mann-Whitney test and the Fisher's exact test. Correlation coefficients given are Spearman's (r_s). Cumulative percentages of response were compared, using the log-rank test. Multivariate Cox regression [Cox, 1972] was used to evaluate various factors simultaneously regarding the cumulative percentage of response. $P = 0.05$ (two-sided) was considered the limit of significance.

RESULTS

HBeAg Assay

Specificity. Serum samples (n = 152) of the control groups of medical students (anti-HBc negative, anti-HBs negative by radioimmunoassay) and long-term chronic hepatitis B carriers without serum HBeAg by IMx were assayed. None was HBeAg positive by AxSYM HBe 2.0 Quantitative.

Reproducibility and precision. Four in-house reference samples were used for inter-assay variation measurements. The percentage coefficient of variation ranged from 4.3% to 16.6%, with the higher coefficient of variation at lower HBeAg levels. Figure 1 illustrates the dilution characteristics of the four in-house reference preparations. No significant deviation from linearity (all $P > 0.26$) was present for any of the preparations. The intra-assay variation was less than 5% (4.48; 4.97) for two samples (seven determinations) at about 64 and 38 PEIU/ml, respectively.

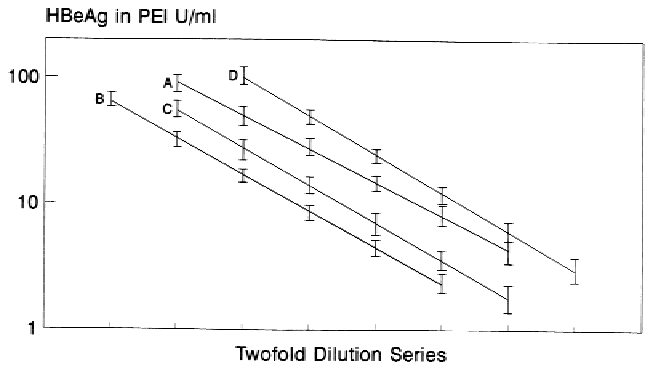

Fig. 1. HBeAg titration curves of four in-house reference samples. Every curve presents mean values and standard deviations from six different runs. Serum samples for separate runs were prediluted (twofold dilution series) with fetal calf serum and stored as single test samples at −20°C until use. For ease of presentation the starting position on the x-axis was chosen arbitrarily. The slope for sample A is less steep as compared to the others ($P < 0.001$). Initial dilutions of the reference samples: A (1 : 10), B (1 : 2.5), C (1 : 2.5), D (1 : 100).

Pretreatment Samples

Dynamic range of the HBeAg assay. Pretreatment samples (n = 89) from 45 patients undergoing interferon or lamivudine treatment were assayed. In general one sample was taken from each patient within 8 weeks before the start of therapy and one sample at the start of therapy.

Forty-one of the 45 (95.5%) patients (85 samples) had HBeAg levels below 6,000 PEIU/ml, which is the upper level of the assay when using the automatic dilution feature. Four pretreatment samples (two patients) had HBeAg levels of 6,000–12,000 PEIU/ml.

In four of the 44 paired pretreatment serum samples, HBeAg levels differed more than fourfold (13- to 300-fold; three patients demonstrated decreases and one showed an increase in samples taken within 6 weeks). In the remaining 40 paired samples, the mean difference between the two specimens was 13.3 ± 12.9%.

HBeAg level distribution. Figure 2 illustrates the HBeAg level distribution before start of treatment in the combined treatment groups. In both groups, most patients had levels of 750–6,000 PEIU/ml; in the IFN group, there was a subgroup with low HBeAg levels.

HBeAg and HBV-DNA. Using geometric mean values of the two pretreatment samples, no significant correlation was found between the pretreatment levels of HBeAg and HBV-DNA ($r_s = 0.18$: $P = 0.23$) in the combined IFN- and lamivudine-treated patients.

Antiviral Treatment

Interferon treatment. Thirty patients were treated with interferon by a standard schedule of 16 weeks, with a follow-up of 36 weeks. Results of HBeAg and HBV-DNA quantitation at the start and end of the study are summarized in Table I. Nine patients responded during the 16-week therapy and five patients during the follow-up period of 36 weeks; all responders were anti-HBe positive at weeks 52–56. Sixteen patients did not have sustained loss of HBeAg during therapy or follow-up.

Predictive value of HBeAg and HBV-DNA quantitation. The level of HBV-DNA before start of therapy was significantly lower in the responder group, compared to the nonresponder group (median levels, respectively, 51 and 186 pg/ml; $P = 0.02$). The difference between the pretreatment levels of HBeAg for the responder and nonresponder groups was not statistically significant ($P = 0.23$).

Twenty-eight of the 30 IFN-treated patients were divided into three groups according to the percentage decrease of HBeAg at week 4, compared to the pretreatment level of HBeAg: group A, reduction of HBeAg >50% (n = 7; range: 81–100%); group B, a decrease with a maximum of 50% (n = 13; range: 5–48%); and group C, no decrease (n = 8, <5%). One patient who responded already within 4 weeks of therapy was excluded from further evaluation. Another patient could not be grouped due to a missing bloodsample at week 4. The cumulative percentage of responding patients increased significantly ($P_{trend} = 0.003$) with a stronger reduction of HBeAg (C → A) (Fig. 3). No responders were found during treatment or follow-up in group C (with no HBeAg decrease at week 4), and all patients in group A responded.

If the result of the HBeAg decline of weeks 0–8 is also taken into account, group B can be further separated into two subgroups: a subgroup of 8 patients with less than 50% HBeAg decrease at week 4 (range: 7–48%; mean: 33%) and more than 50% HBeAg decrease at week 8 (range: 53–99%; mean: 71%) with a response rate of 75% ($P = 0.02$), and a subgroup of five patients with less than 50% decrease of HBeAg at week 4 (range: 5–28%; mean: 16%) and week 8 (range: 7–45%; mean: 27%), and no response during therapy or follow-up.

Using similar analysis for HBV-DNA, it was found that the HBV-DNA decrease at week 4 did not differ significantly ($P = 0.24$) between responders and nonresponders (median decreases, respectively, 71% and 65%).

The pretreatment level of HBV-DNA and the change in HBeAg level from pretreatment at week 4 were evaluated for its predictive value by a multivariate analysis. It was found that the change in HBeAg level was the most important independent predictive factor. No significant additional predictive value was found for pretreatment HBV-DNA.

Lamivudine treatment. Fifteen patients were treated with lamivudine in dosages of 25, 100, or 300 mg/day. Table II illustrates the HBeAg and HBV-DNA changes during therapy. HBV-DNA reduction is almost 100% in all patients. A steep decrease of HBV-DNA (>90% reduction) was observed already in the second-week sample in 12 of 15 patients (results not shown). After cessation of therapy HBV-DNA levels rapidly returned to pretreatment levels in most cases.

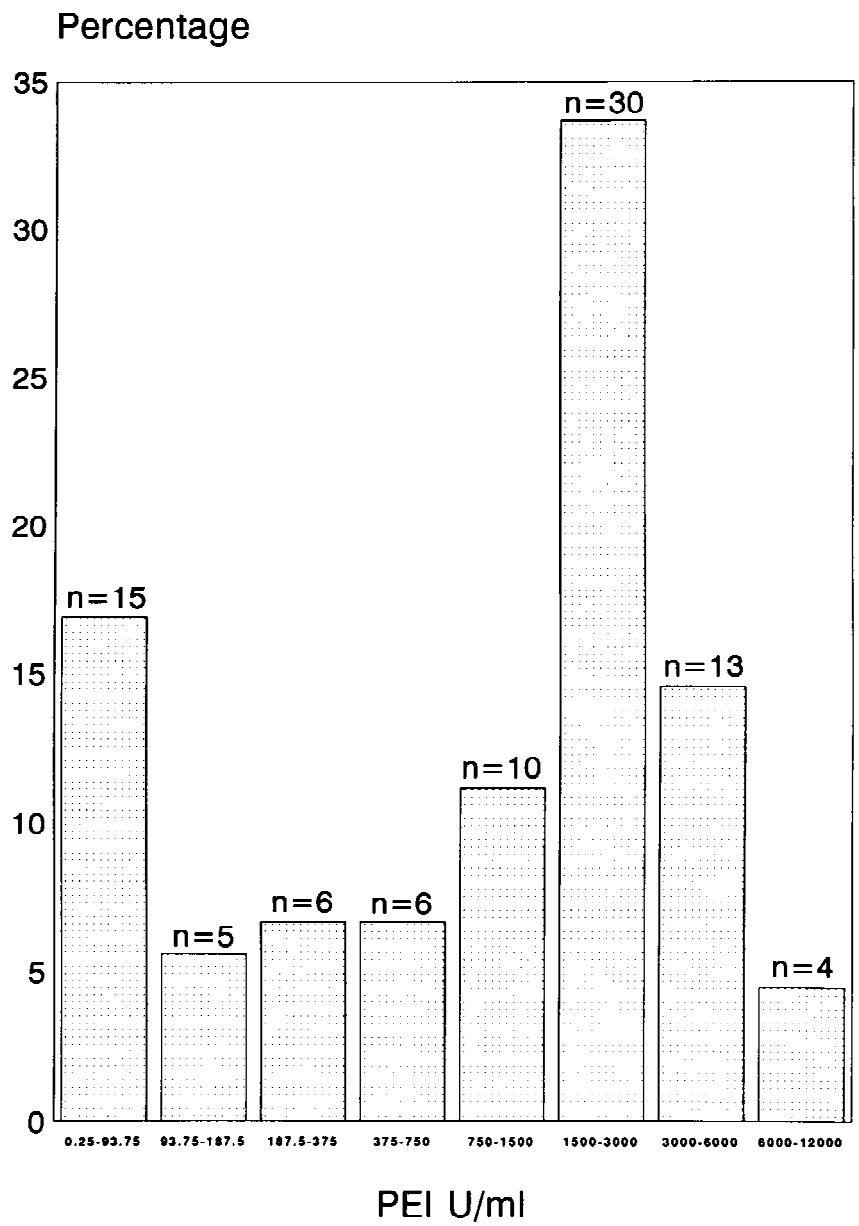

Fig. 2. Distribution of HBeAg levels in PEI U/ml in 89 individual pretreatment samples from 45 patients. Samples were taken within 8 weeks before treatment. n = number of samples.

Reduction of HBeAg levels was observed from week 8 onwards. At that time 3 of the 15 patients (20%) showed a more than 50% reduction of HBeAg. In general, the maximum reduction in HBeAg was reached at the end of therapy (weeks 20–24).

According to our definition of response to therapy (two HBeAg-negative and HBV-DNA <3 pg/ml results on two consecutive samples), two patients (patients 3 and 5) could be classified as responders, one of them (patient 3) seroconverted to anti-HBe, but the other (patient 5) relapsed at the end of follow-up. Patients 3 and 5 showed a 39% and 50% reduction, respectively, in HBeAg level by week 4. In comparison, the fall in HBeAg by week 4 ranged from 0% to 30% in the remaining 13 patients.

DISCUSSION

HBeAg quantitation was carried out for monitoring of antiviral therapy in chronic hepatitis B by an automated microparticle enzyme immunoassay (AxSYM). The AxSYM HBe 2.0 Quantitative combines sensitivity comparable with IMx (results not shown), high reproducibility (intra-assay %CV: 5%; interassay %CV: 4.4–16.6%) and a wide dynamic range (0.25–6,000 PEIU/ml) with ease of performance. In addition, results are presented in a standardized manner.

HBeAg levels were studied from two treatment groups (interferon treatment and lamivudine treatment) and compared to HBV-DNA levels. Serum HBeAg levels from two cohorts before the start of treat-

TABLE I. HBeAg and HBV-DNA Level Changes During Interferon Therapy of 16 Weeks and 36 Weeks of Follow-up

		HBeAg				HBV-DNA				
Pt No.	Cat[a]	Start Rx	Maximum decline (%)	Week[b]	End follow-up	Start Rx	Maximum decline (%)	Week[b]	End follow-up	Resp.[c] (week)
Responders										
1	A	15	100	4	<0.25	45	100	16	4	16
2	A	467	100	8	<0.25	30	100	8	<3	8
3	A	63	100	8	<0.25	41	100	12	<3	44
4	A	62	100	8	<0.25	<3	100	0	<3	52
5	A	4	100	12	0.34	71	100	8	<3	12
6	A	309	100	16	<0.25	7	100	2	<3	16
7	A	70	100	16	<0.25	6	100	4	<3	16
8	B[d]	2,079	100	12	<0.25	113	100	12	<3	12
9	B[d]	2,854	100	16	<0.25	183	100	12	<3	20
10	B[d]	1,878	100	24	<0.25	23	100	4	<3	32
11	B[d]	7,555	100	32	<0.25	262	100	24	<3	32
12	B[d]	2,876	100	44	<0.25	47	100	4	<3	44
13	B[d]	361	100	52	<0.25	59	100	12	<3	52
14	—	2	100	2	<0.25	<3	100	0	<3	2
Nonresponders										
15	B[d]	270	84	52	42	377	71	52	109	—
16	B[d]	1,033	97	32	1,564	31	87	8	51	—
17	B	12	42	36	245	3	100	2	20	—
18	B	1,529	34	12	2,013	145	19	4	276	—
19	B	3,507	34	16	3,041	372	79	2	279	—
20	B	2,500	82	20	1,104	63	100	2	57	—
21	B	1,224	86	52	176	159	84	16	29	—
22	C	3,693	21	12	3,735	288	61	12	1,085	—
23	C	113	99	16	90	318	99	16	38	—
24	C	2,750	54	36	2,552	27	89	32	9	—
25	C	5,784	99	36	50	131	100	32	<3	—
26	C	2,536	85	44	384	223	92	8	18	—
27	C	1,802	98	52	36	374	100	52	<3	—
28	C	821	99	52	1	139	83	28	25	—
29	C	2,263	44	52	1,368	434	48	2	503	—
30	—	1	50	52	<0.5	<3	—		23	—

Pt, patient; Rx, therapy.
[a]Category of patient (A,B,C) corresponding to Figure 3.
[b]First samples with maximal decrease of HBeAg or HBV-DNA since start of therapy.
[c]Response complete according to definition: 2 consecutive samples negative for HBeAg (cutoff 0.25 PEIU/ml) and HBV-DNA (cutoff 3 pg/ml).
[d]Category B with HBeAg reduction >50% at week 8.

ment were very similar in distribution, usually between 750 and 6,000 PEIU/ml. Pretreatment levels of HBeAg were not significantly related to levels of HBV-DNA. In one case (patient 2, Table II), the low level of HBeAg and high level of HBV-DNA suggested the presence of a precore mutant that was confirmed by DNA sequencing (H.G.M. Niesters, personal communication).

At present, monitoring is primarily carried out by measuring HBV-DNA. This study confirms that the level of HBV-DNA falls almost instantly with lamivudine therapy (week 2) and somewhat later during interferon therapy (weeks 2–4), whether or not a sustained response follows. This study also confirms earlier work with a research IMx assay for HBeAg measurement [Perrillo et al., 1993] that HBeAg levels decline more slowly than HBV-DNA.

Furthermore, this study suggests, that quantitative monitoring of HBeAg has the highest predictive value for the outcome of therapy. In contrast to the observation of Perrillo et al. [1993], pretreatment HBeAg was not related to the outcome of therapy. The decline in

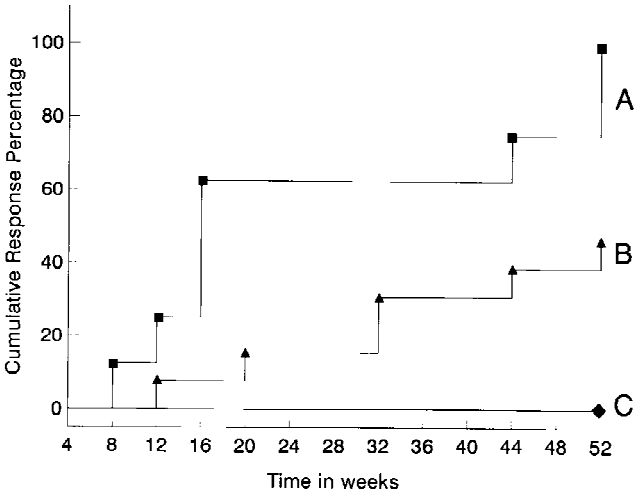

Fig. 3. Cumulative response rate based on the week 0–4 HBeAg level change in serum of 28 patients treated with interferon. A, reduction of HBeAg >50% (n = 7): B, reduction from 0% to 50% (n = 13); C, no reduction of HBeAg (n = 8). Interferon therapy of 16 weeks with 36 weeks of follow-up.

TABLE II. HBeAg and HBV-DNA Level Changes During Lamivudine Therapy of 24 Weeks and 24 Weeks of Follow-up

	HBeAg				HBV-DNA			
Pt/ dose	Start Rx	Maximum decline (%)	Week[a]	End follow-up	Start Rx	Maximum decline (%)	Week[a]	End follow-up
25 mg								
1	1,026	60	24	821	367	100	8	168
2	10	100	20	<0.25[b]	498	99	20	757
3	108	100	24	<0.25[b]	4	100	2	<3
4	1,683	55	16	1,291	12	100	2	6
100 mg								
5	824	100	8	6,147	75	100	4	317
6	4,272	15	24	5,410	108	100	2	115
7	1,718	32	20	1,711	380	99	16	352
8	1,802	41	24	1,622	266	100	12	265
9	5,422	99	24	812	48	100	2	22
300 mg								
10	1,387	81	24	958	95	100	2	85
11	1,972	37	20	2,190	153	97	4	219
12	7,396	0	24	14,224	160	100	4	145
13	2,477	31	16	2,485	214	100	12	187
14	2,403	88	24	2,584	148	100	4	77
15	534	99	48	3	121	100	44	<3

Pt, patient; Rx, therapy.
HBeAg level in PEIU/ml (cutoff 0.25 PEIU/ml; HBV-DNA in pg/ml.
Maximum decline: relative to start of therapy.
[a]Time of lowest level of HBeAg or HBV-DNA.
[b]Anti-HBe positive.

HBeAg by week 4 of IFN therapy identified 50% of patients that would or would not respond on therapy. HBeAg results at week 8 had a strong predictive value in the remaining interferon treated patients.

No additional prognostic information could be ascribed to pretreatment HBV-DNA or the decrease of HBV-DNA during the first eight weeks of interferon. HBV-DNA quantitation will remain of interest to select patients who may benefit from various therapies to lower the viral replication rate—this should be done to detect and quantify the precore mutants.

In conclusion, HBeAg quantitation at weeks 4 and 8 of IFN therapy may have clinical importance in early identification of nonresponders. These results have been obtained in a limited number of patients. Further validation of these findings in another larger cohort is needed before HBeAg quantitation can be used as a definite guideline in assessing whether continued treatment is beneficial.

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